

Study of the effects of spinorphin on painful diabetic neuropathy: A behavioral study in vivo

Spinorphin, diabetic neuropathy

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Abstract

Aim: Diabetic neuropathy is one of the chronic complications of diabetes. Our aim in this study is to examine the effects of spinorphine on painful diabetic neuropathy in vivo.

Material and Methods: Seventy-five three-week rats were used. The first group was determined as a healthy control group (n = 15). Then, diabetic animals were grouped into subassemblies following induction in rats induced streptozotocin and diabetes. Groups were created using rat as follows: First group: Healthy control group, Second group: Diabetic control group (group to be given spinorphine solvent) (n = 15), Third group: DM + SP0.1 group (group to be applied 0.1 mg / kg spinorphine) (n = 15), Fourth Group: DM + SP1 group (group to be applied 1 mg / kg spinorphine) (n = 15) and Fifth group: DM + SP5 group (group to be applied 5 mg / kg spinorphine) (n = 15).

Results: When the diabetic control group and the healthy control group were compared in terms of the pain threshold values, a statistically significant difference was found (P <0.05). This result was considered significant in regards to the development of neuropathy (P <0.05). The pain threshold values in DM + SP0.1 and DM + SP1 groups had no statistically significant differences compared with the diabetic control group (P > 0.05).

Discussion: This study, found spinorphine to be effective at doses of 5mg/kg or higher and when administrated intraperitoneally in the acute antinociceptive treatment of painful neuropathy in diabetic rats.

Keywords

Diabetic neuropathy; Hot plate; Spinorphine

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Introduction

Diabetes mellitus (DM) is a chronic, metabolic disease, which is accompanied by disorders of carbohydrate, lipid and protein metabolism, especially hyperglycemia arising from disorders of insulin secretion, insulin activity, or both, and accompanied by accelerated atherosclerosis and microvascular and macrovascular complications [1, 2].

Various pathogenetic mechanisms play a role in the development of diabetes. These are other causes of pancreas β cell destruction and insulin resistance, which leads to absolute insulin deficiency [3, 4].

Many mechanisms have been proposed in the pathogenesis of diabetic neuropathy. These are metabolic processes that cause direct nerve injury, endoneurial microvascular damage, autoimmune inflammation, and decreased neurotrophic support [5, 6].

Spinorphine is a protein substance that was first isolated from the bovine spinal cord. Spinorphine shows its antinociceptive effect by inhibiting the enzyme fractionation enkephalin [7]. In animal studies related to spinorphine, it has been reported to provide strong antinociception when administered intraventricularly and intrathecally. As a result of these studies, it was found that spinorphine is an important agent in the metabolism of enkephalin in the spinal cord and thus in the modulation of pain [8].

In this study, it was analyzed whether different doses of spinorphine were effective when administered intraperitoneally in the acute antinociceptive treatment of painful neuropathy in diabetic rats.

Material and Methods

Experimental Animals

Approval was obtained from the Animal Experiment Ethics Committee before the commencement of the study. In the experimental study, 75 BALB-C male rats with an average weight of 30 grams (30 ± 5 g) for at least 8-weeks were used. In Experimental Research Unit (FÜDAM) Animal Laboratory of Firat University, the temperature of the environment where the rats are kept is constantly between 22-25°C and the animals were followed during 12 hours of light and 12 hours of darkness. The rats were specially prepared in an environment with a ventilation system and were fed in cages cleaned every day. Feed was given in a special steel trough, and water was given as normal tap water in stainless steel ball feeding bottles. The experimental animals were fed with specially prepared rat feeds in the form of pellets.

Diabetes induction

In this part of the study, 150 mg/kg STZ (Streptozocin, Zanosar, Pharmacia, France) by insulin injection of 26 gauge because of the induction diabetes in 60 rats was administered as a single dose by intraperitoneal (ip) injection [9] solubilized (pH: 4.5) in 0.4 ml (0.1M) sodium-citrate buffer. One week later, rats whose postprandial blood glucose exceeded 400 mg/dl as a result of measurement on the glucometer device by being taken blood from the tail vein were considered diabetic [10]. ACCU-CHEK Go (Roche) was used as a blood glucose meter. A drop of fresh blood taken from the tail of the experimental animal was absorbed into the strip of the blood glucose meter, and then,

after 10 seconds, the blood glucose level was displayed on the device screen. Measurement of blood glucose level on these devices was performed by the "glucose-oxidase peroxidase" method [11].

Streptozocin is protected from light because it has the structure of N- (Methylnitrosocarbamoyl) - α -D-glucosamine. The pH of the environment was kept at 4-4.5 for optimum stability since it has decomposed rapidly at neutral pH. Therefore, while solubilising streptozocin, citrate buffer was used. Both insulin-dependent and insulin-independent diabetes have been induced by injuring pancreatic β cells [12].

Experimental Groups

The study consists of 5 groups.

1st group (n = 15): Healthy control group.

Following induction in rat induced diabetes using STZ (approximately 4th week); diabetic animals were separated into subgroups (n = 60).

2nd Group (n = 15): Diabetic control group (the group to be administered with spinorphine solvent).

3rd Group: DM + SP0.1 group (n = 15): The group to be administered low doses of spinorphine (Spinorphine with 0.9% saline at a dose of 0.1 mg/kg by solubilising within the physiology was administered by injecting).

4th Group: DM + SP1 group (n = 15): Group to be administered spinorphine at a median dose (Spinorphine with 0.9% saline at a dose of 1 mg/kg by solubilising within the physiology was administered by injecting).

5th Group: DM + SP5 group (n = 15): Group to be administered the maximum dose of spinorphine (Spinorphine with 0.9% saline at a dose of 5 mg/kg by solubilising within the physiology was administered by injecting).

Hot Plate Test in Diabetic Rats

Pain threshold values in study and control animals were analyzed using the hot plate test two weeks after the induction of diabetes. The ground temperature of the hot plate analgesimeter was set to 50 ± 0.5 °C to create thermal hyperalgesia. While the animal was kept in this closed area, the chronometer was started. Observing the experimental animal, when forming a reaction such as pulling its nails, preening its nails due to pain, or jumping, the chronometer was stopped and withdrawal latency value was obtained in seconds.

Before starting pain threshold studies, animals were enumerated by means of marking the tails of the animals in all groups. First of all, control records of all groups were taken 15 minutes before injection and the time of injection was considered as 0 minutes. Following the injection, pain threshold values were measured at 15th, 30th, 45th, 60th, 120th and 180th minutes. It was taken care that the people who performed antinociceptive behavioral experiments did not have information about the blood glucose level of the rat.

Statistical Analysis

The average and standard deviation values of the data obtained in the study were calculated. SPSS 22.00 computer package statistics program (SPSS Inc., Software Chicago, IL, USA) was used in preparation of statistics. The Kruskal-Wallis and Mann-Whitney U tests were used in the analysis. A p-value <0.05 was considered significant.

Results

Before starting the experimental studies, in order to minimize the problems that may occur, all groups underwent a period of adaptation to the environment and hot-plate test for a week. Initially, pain threshold values were determined before the rat was not injected. Following these procedures, the animals in the control group were injected with saline, and (n = 15) physiological study group, respectively; Low doses of spinorphine (0.1mg/kg), median dose spinorphine (1mg/kg) and maximum dose spinorphin (5mg/kg) were administered by injection. The time of injection was accepted as 0 minutes. Since the half-life of spinorphine ($t_{1/2}$) is 20 min, pain threshold values in all groups were measured with a hot plate device at 0th, 15th, 30th, 45th, 60th, 120th and 180th minutes, and their records were taken.

Pain threshold values of the healthy control group, were as follows: -15th minute: 16.18 ± 0.3, 0th minute: 16.55 ± 0.30, 15th minute: 17.25 ± 0.34, 30th minute: 16.89 ± 0.13, 45th minute: 15.48 ± 0.17, 60th minute: 17.67 ± 0.17, 120th minute: 16.09 ± 0.24 and 180th minute: 15.91 ± 0.20 seconds (Table 1). Pain threshold values of the diabetic control group, were as follows: -15th minute: 22.95 ± 0.88, 0th minute: 23.62 ± 0.97, 15th minute: 23.10 ± 0.84, 30th minute: 23.52 ± 0.97, 45th minute: 23.62 ± 0.69, 60th minute: 22.25 ± 0.89, 120th minute: 24.02 ± 0.83 and 180th minute: 23.76 ± 0.91 seconds (Table 1). A statistically significant difference was found when comparing the pain threshold values between the healthy control group and the diabetic control group, ($p < 0.05$).

Pain threshold values in the DM + SP0.1 group, were recorded as follows: 22.36 ± 1.14, 22.25 ± 1.11, 21.82 ± 1.02, 20.48 ± 0.10, 21.35 ± 1.13, 21.05 ± 0.84, 21.15 ± 0.98, 21.13 ± 0.73 and 20.55 ± 0.38 seconds (Table 1). Pain threshold values in the DM + SP1 group, were determined as follows: 22.42 ± 0.80, 21.79 ± 0.94, 22.43 ± 0.94, 22.77 ± 1.13, 22.16 ± 0.95, 21.71 ± 0.85, 21.63 ± 0.92 and 21.38 ± 0.76 seconds (Table 1). Pain threshold values in DM + SP5 group, were determined as follows: 20.02 ± 1.16 sec, 20.58 ± 0.86 sec, 21.33 ± 0.22 sec, 23.12 ± 0.96 sec, 23.22 ± 0.10 sec, 21.53 ± 0.77 sec, 21.31 ± 0.83 sec and 21.44 ± 1.45 sec (Table 1). When comparing the pain threshold values measured at 30 and 45 minutes in the DM + SP5 group with the pain threshold values at -15, 0, 15, 60, 120 and 180 minutes, a statistically significant difference in acute antinociceptive effect on painful diabetic neuropathy was found ($p < 0.05$).

Discussion

The hot plate test in the evaluation of neuropathic pain threshold is an indirect and in vivo method and the evaluation is made quantitatively. It has been reported that pain threshold reactions contribute to obtaining indirect information about diabetic neuropathic pain [13, 14,15].

The fact that it has an antinociceptive effect against painful stimuli has indicated the necessity of testing the activity possibilities of this agent in the acute treatment of neuropathic pain. For this reason, there are various studies in the literature [16-17].

Spinorphine and other enkephalin hydrolyzing enzyme inhibitors are highly promising therapeutic agents for antinociceptive therapy. Especially in recent years, the antinociceptive effects of these agents have been examined and reported. The most important advantages of these antinociceptive agents are that both are stronger than opioids and do not have side effects caused by opioids [18].

In a study carried out by Honda et al. [19], the effects of spinorphine on allodynia on thermal and mechanical nociception in rats in vivo were investigated. They demonstrated that intrathecal administration of spinorphin in a dose-independent manner inhibited intrathecal nociception causing allodynia. In addition, they found that spinorphine caused prolongation of the antinociceptive effect created by leu-enkephalin when administered intracerebroventricularly. Still in this study, they applied spinorphine intracerebroventricularly and all in all found that spinorphine did not change both the mechanical and thermal pain threshold. In this study, they stated that when administered intrathecally, spinorphin exerts its antinociceptive and antiallodynic effect by inhibiting the enzymes that break down enkephalin.

Nishimura et al. [20] administered spinorphin in to the monkey brain at 50-200 mcg doses intraventricularly in an animal study they conducted. At the end of the study, they found that spinorphine showed antinociceptive activity in a dose-independent manner. They considered from this effect of spinorphine to inhibit enzymes that hydrolyze enkephalin with a high affinity and potently, and they expressed that spinorphine is an important neuromodulator for enkephalin metabolism in the spinal cord [20].

Jung et al. [21] demonstrated that the antinociceptive effect of spinorphine, an endogenous peptide, is associated with

Table 1. The rat pain threshold values in the control group DM, DM + SP0.1, DM + SP1, DM + SP5 with hot-plate test

Groups (n = 15)	15 th min.	0 th min.	15 th min.	30 th min.	45 th min.	60 th min.	120 th min.	180 th min.
Control	16.18 ± 0.32	16.55 ± 0.30	17.25 ± 0.34	16.89 ± 0.13	15.48 ± 0.17	17.67 ± 0.17	16.09 ± 0.24	15.91 ± 0.20
DM Control Group	22.95 ± 0.88	23.62 ± 0.97	23.10 ± 0.84	23.52 ± 0.97	23.62 ± 0.69	22.25 ± 0.89	24.02 ± 0.83	23.76 ± 0.91
DM + SP0.1 Group	22.36 ± 1.14	22.25 ± 1.11	21.82 ± 1.02	20.48 ± 0.10	21.35 ± 1.13	21.05 ± 0.84	21.15 ± 0.98	21.13 ± 0.73
DM + SP1 Group	22.42 ± 0.80	21.79 ± 0.94	22.43 ± 0.94	22.77 ± 1.13	22.16 ± 0.95	21.71 ± 0.85	21.63 ± 0.92	21.38 ± 0.76
DM + SP5 Group	20.02 ± 1.16	20.58 ± 0.86	21.33 ± 0.92	23.12 ± 0.96	23.22 ± 0.10	21.53 ± 0.77	21.31 ± 0.83	21.44 ± 1.45

± = standard deviation

inhibition of the enzymes that hydrolyze enkephalin, in particular, non-competitive and selective antagonism at the ATP-dependent human P2X3 receptors.

Yamamoto et al. [7] applied spinorphine intraventricularly to the monkey brain in a study they conducted. As a result of the study, they considered the antinociceptive effect of spinorphine in inhibiting the destruction of enkephalin. They showed that enkephalin has played a role in a highly complex pain modulation mechanism in the spinal cord. They reported that enkephalin is rapidly fractionated by endogenous enzymes and therefore it is ephemeral; spinorphin shows its antinociceptive effect by inhibiting the enzymes that hydrolyze enkephalin, especially DPP III. There are very few studies in the literature regarding the antinociceptive effect of spinorphine, and in these studies [7, 8, 19], they applied spinorphine to the monkey brain intraventricularly and intrathecally in a total dose range of 50-200 mcg. and the antinociceptive effect was obtained after thirty minutes. In this study, spinorphine was administered at a dose of 5 mg/kg as the highest dose and intraperitoneally. It has been determined that spinorphine does not show the antinociceptive effect at low doses, such as 0.1mg/kg and 1mg/kg and when administered intraperitoneally. However, when administered at a high dose such as 5mg/kg, it showed an antinociceptive effect when measured after 30 and 45 minutes ($p < 0.05$). When applied at this dose, the antinociceptive effect was observed at 60, 120 and 180 minutes ($p > 0.05$). In accordance with the results obtained in previous studies on spinorphine, and also, in our study, the antinociceptive effect was obtained when administered at high doses. The half-life of spinorphine of 20 minutes has explained the observation of antinociceptive effect at 30 and 45 minutes when administered at a dose of 5 mg/kg and the termination of antinociception after 60 minutes.

In current studies on spinorphine, spinorphine was administered centrally such as intraventricular and intrathecal administration. Intraperitoneal administration was tried for the first time in this study, and a positive response was obtained in high dose administration. This result is important for the effectiveness of peripheral administration of spinorphine.

Limitations

Our study has several limitations. A wider group of rats could be used. Spinorphine could be given after a certain period of time after diabetes developed in rats. Thus the effect of complications could be seen more clearly. However, as rat losses may increase in this condition, it was not considered appropriate. A comparison of spinorphine with another molecule actively used in the treatment of diabetic neuropathy could be done. Thus, we could see the effect of spinorphine on diabetic neuropathy more clearly. However, the current situations are not included in the project due to high costs.

Conclusions

In conclusion, in this study, it was determined that spinorphine was effective in the acute antinociceptive treatment of painful diabetic neuropathy. To induce an acute antinociceptive effect of spinorphine, it should be used in doses of at least 5mg/kg or higher. There is no antinociceptive effect at lower doses. The antinociceptive effect appeared as of 30 minutes and disappeared after 60 minutes. In consideration of all these

findings, spinorphine is seen as a promising agent for the acute antinociceptive treatment of painful diabetic neuropathy.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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